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"Effects of High and Low Barometric Pressures on Susceptibility and Resistance to Infection"

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Abstract.

The pulmonary infection of mice induced by exposure to an aerosol of the chlamydial mouse pneumonitis agent was definitely reduced in degree by post-challenge exposure to a hyperoxic environment (77% O_2 in N_2 at 1 atmosphere), and evidence was obtained that hypoxic (11% O_2 in N_2 at 1 atmosphere) enhanced the infection. Both of these observations confirm or are compatible with previous findings.

When Chlamydia psittaci is injected i.p., the resulting infection is increased in severity when the mice are exposed to hypoxia. A simulated space cabin atmosphere (70% O_2 in N_2 at 5 psia) had no effect on the mortality curve, when compared to air-exposed controls, with this infectious test-system.

Using exposure of mice to an aerosol of influenza virus as the test system, experiments demonstrated a protective effect of hypoxia applied only before the infectious challenge. This confirms early investigations in this field by others. A minimal difference in pathologic changes, but not in viral titer of lung tissue was detected between the test and control groups. Although the mice may have been sacrificed at an unsatisfactory interval. Exposure to a simulated space cabin atmosphere (70% O_2 in N_2, 5 psia) resulted in a greater mortality in the test mice than in the controls, the same effect seen earlier with 100% O_2 at 3.3 psia (ambient pO_2, 160 mm Hg).

Great variation in the numbers of lung tumors seen in A/HeJ mice following induction with dibenz-anthracene continues to give trouble. This fact probably contributes to the insignificant differences seen between groups in the last experiment of this time.

The status of new chambers for more precise control of parabaric environments is reported.

1. Effect of parabarosis on infection of mice with Chlamydia trachomatis (mouse pneumonitis strain).

Experiment Mopn #39 was designed to determine the effect on chlamydial pulmonary infection in mice of post-challenge exposure to hyperoxia and hypoxia, both in normobaric (1 atmosphere) conditions. The mice were exposed to an infective aerosol in the usual manner on day 0, and then placed in the test environments. The two parabaric environments were 77% O_2 in N_2, 11% O_2 in N_2. These mixtures, and tank air for the control groups, flowed through the one-atmosphere chambers containing the mice. Two groups of 10 or 11 mice each were used for each kind of environment, one for observation of mortality rates, the other for sacrifice and titration of lung tissue.

The results are presented in Table 1. Although the aerosol used was less infective than calculated (due to a mechanical defect discovered later), resulting in insignificant mortality rates, observations on the sacrificed mice were informative. The four factors assessed, weight of mouse, weight of lung, degree of consolidation, and infective titer of lung (IFU/ml), all point to a protective effect of 77% O_2 in comparison to the other two groups. This confirms and extends the results seen in earlier tests (see Table 1 of QSR #7, 31 March 1967).

The results with mice under hypoxia (11% O_2 at one atmosphere strongly suggest a somewhat greater degree of infection, particularly evident when one compares the two 14-day sacrificed groups of mice. This result is compatible with that of Exp. Mopn #36 (see Table 1 of QSR #13, 30 Sept 1968) in which the hypoxia was induced by simulated altitude (air at 7.3 psia).

2. Effect of parabarosis on mice infected with Chlamydia psittaci.

Mortality rates observed in C. psittaci-infected mice subjected to either simulated altitude with normal pO_2 (37,000 ft, 100% O_2) or hypobaric-hypoxic atmospheres (18,000 ft, pO_2 80 mm Hg) have been described in QSR's, No. 12 and 13. Either post-challenge or both pre- and post-challenge exposure to altered atmospheres were utilized. Evidence that departures from the normal host-agent effect are primarily dependent upon alteration of the parabaric conditions following, rather than preceding, challenge was presented in QSR No. 14 (Exp. Psitt #6), when hyperoxic-normobaric (77% O_2 at 1 atm) and corresponding flowing line air and shelf control animals were utilized. The reduced mortality observed in the hyperoxic groups was as pronounced in animals exposed post-challenge only, as in those exposed both pre- and post-challenge. On this basis, experiment Psitt #7, utilizing a simulated space cabin atmosphere (70% O_2 in N_2, 5 psia), a hypobaric-normoxic atmosphere (18,000 ft, pO_2 80 mm Hg), and line air controls, 1 atm, was designed to determine the effect of only post-challenge exposure to the parabaric environment. Ten mice were used in each group.

As before the i.p. challenge consisted of 0.25 ml of a 2×10^{-5} dilution in phosphate buffered saline (one LD_{50} dose) of C. psittaci, strain 6BC - #3305. Survival rates for each parabaric group and controls are presented in Figure 1.

The effect of post-challenge exposure to hypoxia is again clearly indicated by the decreased survival time and 100% mortality observed in mice (Group C) subjected to the hypobaric-hypoxic environment. The same effect was seen in earlier experiments (Psitt #1 and #2) reported in QSR's #12 and #13.

The results (Group B) with simulated space-cabin atmosphere (70% O_2 in N_2, 5 psia) are the first reported, using this host-microbe model. It is reassuring that the survival curve seen in mice under this condition is almost exactly the same as that of the controls. This result is in contrast to the greater mortality seen in mice exposed to an aerosol of influenza virus and placed in 70% O_2 in N_2, 5 psia (Exp. PR-8 #10, see below), and emphasizes the need for attention to pulmonary infections in parabaric conditions. Comparison of this result, using i.p. injected C. psittaci and that of aerosol-induced chlamydial infection (mouse pneumonitis strain) in a similar environment, must await performance of additional experiments. Although we have assumed that our regularly used hypobaric-normoxic environment (100% O_2, 3.3 psia) was closely similar to the 70% O_2 in N_2, 5 psia, atmosphere, an assumption supported by the similar results with influenza viral infection in the two types of environment, the results of the current experiment, Psitt #7, differ markedly from those of Psitt #1, in which the 100% O_2 environment appeared to provide some protection from i.p.-induced chlamydial infection in contrast to the greater mortality of mice in this environment following aerosol exposure to chlamydiae (mouse pneumonitis). This question, too, can be resolved only by additional experiments.

3. Effect of parabarosis on pulmonary infection of mice with influenza virus.

Three experiments have been performed in which groups of mice were placed in parabaric environments before or after exposure to selected doses of influenza virus (PR-8) in an aerosol. Two groups of 10-12 mice were placed in each of the environments, one group for observation of mortality, and the other for sacrifice at intervals, observation and titration of lungs.

In Exp. PR-8 #9 the test mice were exposed to parabaric conditions before infection. They were placed in chambers with flowing air at 7.3 psia (pO_2, 80 mm Hg; simulated 18,000 ft altitude), i.e., in hypobaric, hypoxic conditions, and the controls in similar chambers in air at one atmosphere (normobaric, normoxic condition). After 2 weeks they were removed from the chambers, subjected to the aerosol challenge, and returned to a shelf in the animal room (normobaric, normoxic conditions).

Figure 2 depicts the survivor-curve of PR-8 #9, and it may be seen that mortality in the control group was 70% (7/10 mice). In contrast, 2 only of the test group of mice died. This result is similar to that reported for mice exposed to influenza virus aerosol and subsequently placed in a hypobaric-hypoxic chamber (Exp. PR-8 #3, Fig. 5, QSR #12), and directly confirms the earlier experiments of Kalter, et al, 1952, 1955, and of Berry, et al, 1955, cited in QSR #12.

Lungs were harvested from test and control groups on days 4, 5, 6 and 7, as indicated in Fig. 2, and the observations on these harvests are recorded in Table 2. These intervals were not optimal and this experiment, and probably not satisfactory, as previous evidence indicates that the highest viral titers are found in the lungs just before the mice begin to die. In this experiment the first death did not occur until the 11th day, and there is little or no evidence in Table 2 for a significant difference between the test and control groups.

In Exp. PR-8 #10 two parabaric conditions were used only after aerosol challenge, a simulated space cabin atmosphere (70% O_2 in N_2, 5 psia, and tank air at 7.3 psia, as in the preceding experiment. Figure 3 indicates the results of observations on survival. The space-cabin atmosphere (Group B) had the same effect, i.e., increased mortality, as observed before in our regular hypobaric, normoxic environment (100% O_2 at 3.3 psia). The latter result was observed in Exp.'s PR-8 #3, #4, and #8 in QSR's #12 and 14.

Again the hypobaric, hypoxic environment (Group C) exerted a protective effect, confirming previous experiments described and cited above.

Although not indicated in Fig. 3, lung harvests were taken from parallel groups of mice on days 4, 5, 6, 7, and the usual observations were made (Table 3). Again, the last lung harvest occurred 3 days before the first death in the parallel group and interpretation of the data in Table 3 is difficult. There is very little evidence of differences among the groups except when the average degree of lung consolidation for entire groups is calculated. The lung score for Group C, in which the parallel group had the least mortality, was about one-half that of the control, Group D, as well as that of Group B.

The mice of Exp. PR-8 #11 were held at one atmosphere pressure, but the 3 groups were subjected to hyperoxia (77% in N_2), hypoxia (11% in N_2) and to air (controls) before aerosol challenge. They were then put on the shelf. The challenge dose of aerosol was less than predicted (a mechanical deficiency) and mortality was negligible. The slight differences observed (recorded in Table 4) cannot be regarded as significant compatible with what has been seen before, Group C, exposed to hypoxia, appeared to have definitely less lung consolidation than the other 2 groups.

4. The effect of parabarosis on induction of pulmonary tumors in A/HeJ strain mice following i. v. challenge with chemical carcinogen.

The cumulative loss of A/HeJ mice in hypobaric-normoxic or hypobaric-hypoxic environments, following induction by dibenz-anthracene, made statistical evaluation impossible (QSR #14, Dec., 1968). Similar parabaric conditions were again employed in the present experiment. Equal weight groups of 14 or 15 mice each were again exposed to the various parabaric conditions, as recorded in Table 5 immediately following i. v. injection of 0.1 mg of dibenz-anthracene.

After exposure to the various altered atmospheres for the times indicated, the mice were held in ambient room atmosphere for four months and then sacrificed under anesthesia. The lungs were infiltrated in situ and tumor nodules counted as before.

Although all animals in the various experimental groups survived in this instance, the resulting incidence of lung tumors observed at sacrifice was very erratic within each experimental group. As indicated in Table 5, the mean value for tumor incidence in each group is generally low but individual animals ranged from no tumors present to over 50 in some instances. This is reflected in the large standard errors calculated for each parabaric group. No inference of alteration in tumor incidence can be made at the level of significance calculated by Student's t in comparing the various parabaric groups.

Steps have been taken to control more vigorously several factors possibly responsible for the within-group variations that have occurred in this type of experiment. If this difficulty cannot be overcome in experiments now underway, this line of inquiry will be abandoned.

5. New equipment.

A shipping accident has again delayed delivery of two additional animal chambers that will allow more extensive experiments both at simulated altitude and depth. These will be delivered in April, 1969. In the meantime, the one chamber of this type has been employed for exploratory short-term tests. Present evidence indicates that these chambers will indeed extend the range of parabaric conditions that can be satisfactorily investigated.

Also plans are underway to construct similar chambers, but without the capacity to allow marked departures from normal pressures. These will serve to house the control animals, thus allowing more efficient use of the pressure chambers only for increased or decreased pressures.

A few observations have been made on temperatures of mice taken to simulated altitude, as projected in the former QSR. These will be continued with new thermistors designed for use in the new chambers.

Table 1. Experiment Mopn #39. Effect of post-challenge parabarosis on mouse lung infection with C. trachomatis (strain mouse pneumonitis)

Environment (1 atm pressure)	Mortality	Observations on sacrificed mice				
		Day of sacrifice*	Av. mouse wt. (gm)	Av. lung wt. (gm)	Lung consol.	Lung titer IFU/ml($\times 10^4$)
77% O_2 in N_2	0/11	11	21.8	0.26	0.8** (3, 1, 0, 0, 0)	5.2 ± 0.1
		14	24.5	0.20	0.06 (0.3, 0, 0, 0, 0)	1.5 ± 0.6
11% O_2 in N_2	1/11	11	17.3	0.34	1.7 (4, 3, 1, 0.5, 0)	92 ±19
		14	16.4	0.41	2.4 (4, 3, 2, 2, 1)	259 ±18
Air (control)	1/10	11	17.9	0.26	1.5 (4, 1, 1, 1, 0.5)	64 ± 4
		14	18.3	0.36	1.3 (3, 2, 1, 0.5, 0)	36 ± 3.5

* 5 mice were sacrificed in each group.

** Average score, and (individual scores) on an arbitrary 0-5 scale.

IFU = Inclusion-forming units.

Table 2. Exp. PR8-9. Effect of two weeks pre-challenge parabaric exposure with aerosol of PR-8 influenza virus on mouse lung infection.

Environment	Day of sacrifice post-challenge†	No. of mice	Av. lung wt. (gm)	Lung consol.*	Infectivity titer of lung (EID_{50};Kärber)
	4	2	0.20	0.0 (0, 0)	$10^{5.5}$
Group B					
	5	3	0.24	0.0 (0, 0, 0)	$10^{6.5}$
Tank air,					
7.3 psia	6	3	0.28	1.7 (2, 2, 1)	$10^{6.3}$
	7	3	0.28	1.3 (2, 1, 1)	$10^{5.8}$
	4	2	0.26	0.0 (0, 0)	$10^{4.6}$
Group C					
	5	3	0.25	0.7 (0, 1, 1)	$10^{5.7}$
Line air,					
1 atmosphere	6	3	0.27	1.3 (2, 1, 1)	$10^{6.5}$
	7	3	0.27	1.7 (2, 2, 1)	$10^{6.3}$

* Arbitrary 0-5 scoring for degree of lung involvement.

† Aerosol exposure. 20 minutes using 1:100 dilution of PR-8, pool A, at 0.3 ml/min, RH 93% .

Table 3. Exp. PR8-10. Effect of parabaric conditions on mouse lung infection following aerosol challenge with PR-8 influenza virus.

Environment	Day of sacrifice, post-challenge†	No. of mice	Av. lung wt. (gm)	Lung consolidation: Individual scores*	Lung consolidation: Av. of group(12)	Infectivity titer of lung (EID_{50};Kärber)
Group B	4	3	0.25	0.0 (0, 0, 0)		$10^{7.1}$
70% O_2 in N_2 5 psia	5	3	0.24	0.0 (0, 0, 0)	0.83	$10^{7.3}$
	6	3	0.33	1.25 (2, 1, 1)		$10^{7.2}$
	7	3	0.31	2.0 (3, 2, 1)		$10^{7.9}$
Group C	4	3	0.22	0.0 (0, 0, 0)		$10^{6.9}$
Tank air, 7.3 psia	5	3	0.22	0.0 (0, 0, 0)	0.42	$10^{7.2}$
	6	3	0.26	0.5 (0.5, 0.5, 0.5)		$\geq 10^{8.3}$
	7	3	0.27	1.2 (2, 1, 0.5)		$> 10^{8.5}$
Group D	4	3	0.25	0.0 (0, 0, 0)		$10^{7.3}$
Tank air control 1 atm	5	3	0.21	0.7 (1, 1, 0)	0.92	$10^{7.1}$
	6	3	0.28	0.7 (1, 0.5, 0.5)		$> 10^{8.5}$
	7	3	0.26	2.3 (3, 2, 2)		$\geq 10^{8.3}$

* Arbitrary 0-5 scoring for degree of lung involvement.

† Aerosol exposure. 20 minutes using 1:100 dilution of PR-8, pool A, at 0.31 ml/min, RH 80%.

Table 4. Exp. PR8-11. Effect of pre-exposure to altered atmospheres on mouse lung infection following aerosol challenge with PR-8 influenza virus.

Environment (pre-challenge)	Mortality	Observations on sacrificed mice					
		Day of sacrifice (post-challenge)	No. of mice	Av. mouse wt. (gm)	Av. lung wt. (gm)	Lung consol.	Infectivity titer of lung (EID_{50};Kärber)
	2/10	4	3	18.2	0.20	0.1 (0, 0, 0.3)	$10^{7.1}$
Group B		5	3	18.3	0.20	0.4 (0.6, 0.3, 0.3)	$10^{7.0}$
77% O_2							
1 atm		6	3	16.8	0.21	1.2 (2, 1, 0.6)	$10^{7.0}$
		7	3	21.0	0.28	1.5 (2, 1.5, 1)	$10^{6.5}$
	1/9	4	3	15.7	0.18	0.2 (0, 0, 0.6)	$10^{7.7}$
Group C		5	3	14.8	0.18	0.2 (0.3, 0.3, 0)	$10^{6.9}$
11% O_2							
1 atm		6	3	16.2	0.18	0.3 (0.6, 0.3, 0)	$10^{6.7}$
		7	2	14.0	0.21	0.7 (1, 0.3)	$10^{6.5}$
	0/10	4	3	20.5	0.19	0.2 (0.3, 0.3, 0)	$10^{6.5}$
Group D		5	3	18.8	0.21	0.4 (0.6, 0.6, 0)	$10^{6.5}$
Air control							
1 atm		6	3	18.3	0.22	0.7 (1, 0.6, 0.6)	$10^{6.7}$
		7	3	20.7	0.27	2.3 (3, 2, 1.5)	$10^{6.0}$

Table 5. Pulmonary adenomas in A/HeJ mice subjected to parabaric environments following i.v. injection of dibenz-anthracene.

Group	No. mice at challenge	Type and duration of environment	No. mice at sacrifice	Average No. tumors
B	15	100% O_2, 3.3 psia 2 weeks	15	4.5 (±) 0.9
C	15	Tank air, 7.3 psia 2 weeks	15	3.3 (±) 1.0
D	14	11% O_2 in N_2, 1 atm 2 weeks	14	4.7 (±) 1.3
E	14	Tank air, 1 atm 2 weeks	14	7.4 (±) 3.7
F	14	100% O_2, 1 atm 48 hours	14	6.1 (±) 3.8

Level of significance by Student's t; t_{27} - C-E: $0.3 > P > 0.1$

P values for all other experimental groups approach 1

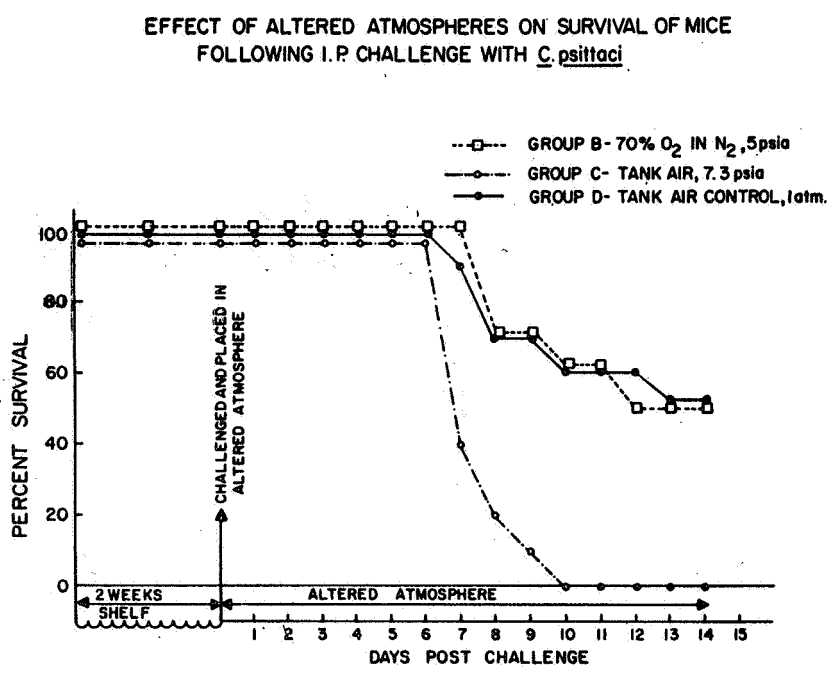

Figure 1. Exp. Psitt-7. Strain 6BC injected in an estimated dose of 1 LD_{50}.

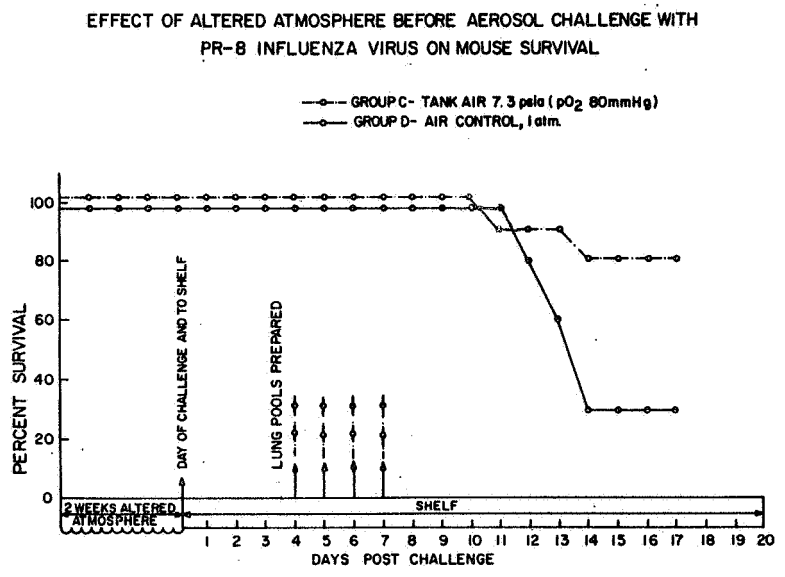

Figure 2. Exp. PR8-9.

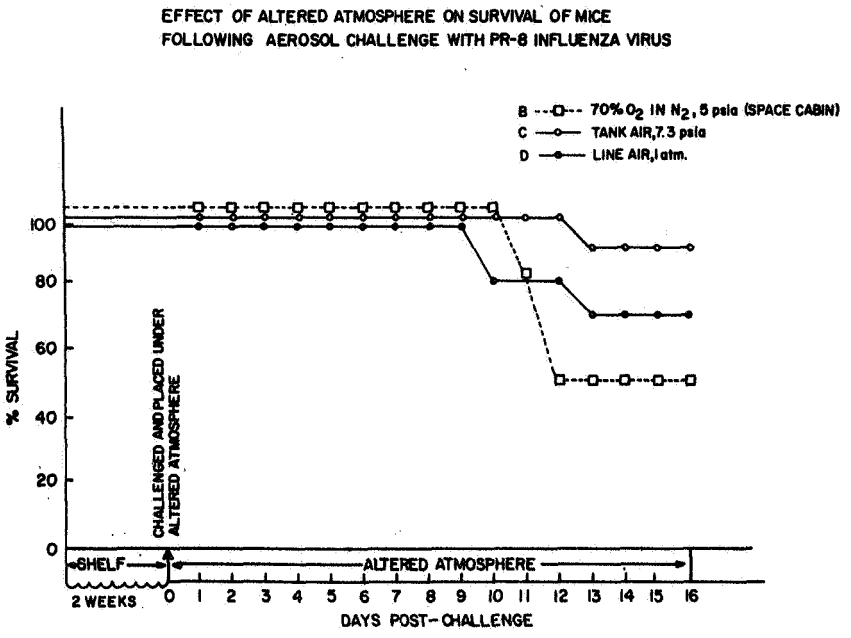

Figure 3. Exp. PR8-10.

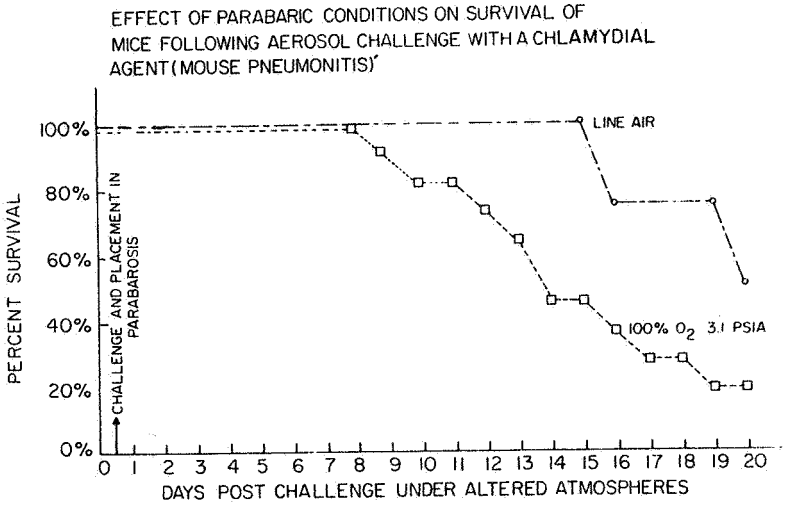

Figure 19. C. trachomatis pulmonary infection at simulated altitude (36,000 ft) and 100% O_2.

EFFECTS OF ALTERED ATMOSPHERES ON MORTALITY RATES OF EXPERIMENTAL MICE

DISEASE AGENT	HYPEROXIA (77% O_2, 1 ATM)	HYPOXIA (AIR, 7.3 PSIA, or 11% O_2, 1 ATM)	100% O_2, 3.2 PSIA
INFLUENZA VIRUS	↗	↘	↗
C. TRACHOMATIS (mouse pneumo.)	↘	↗	↗
C. PSITTACI	↘	↗	
COXSACKIE B VIRUS	→	↗	

↗, ↘, →, MORTALITY INCREASED, DECREASED, OR NOT CHANGED.

Figure 20. Effects of altered atmospheres on mortality rates of experimental mice.